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Assessment of radiation sensitivity of fresh-cut vegetables using electrolyte leakage measurement

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Abstract

A study was carried out to assess the use of electrolyte leakage measurement to evaluate radiation sensitivity of 13 fresh-cut vegetables, and correlated radiation sensitivity with endogenous antioxidant capacity. Fresh-cut vegetables were gamma irradiated at doses up to 3 kGy at 0.5 kGy intervals. Electrolyte leakage of the samples was measured following irradiation. Electrolyte leakage increased linearly with higher radiation dose for all vegetables. The radiation sensitivity, judged from the rates of the increase in electrolyte leakage as a function of radiation doses and from the doses that increased electrolyte leakage by 50% over the non-irradiated controls varied among vegetables. Red cabbage, broccoli and endive had the highest radiation resistance while celery, carrot and green onion were the most sensitive to radiation. The radiation sensitivity was not necessarily correlated with endogenous antioxidant capacity or phenolics content of the vegetables, which showed large variation among the test samples. Electrolyte leakage may be a useful tool to predict a given product's ability to tolerate irradiation.

Keywords: Irradiation; Electrolyte leakage; Fresh-cut vegetables; Radiation sensitivity; Antioxidants

1. Introduction

Ionizing radiation is a non-thermal technology that eliminates food-borne pathogens and extends shelf-life of fresh fruits and vegetables (Thayer and Rajkowski, 1999). In foods (such as fresh vegetables) that contain predominantly water, ionizing radiation exerts its effects through free radicals generated from radiolysis of water. These free radicals (mainly hydroxyl radicals and hydrated electrons) attack biomolecules such as lipids in cell membranes, membrane associated enzymes and proteins, resulting in malfunction of membrane.

Cellular membranes are composed of proteins associated with a lipid bilayer matrix (Voet and Voet, 1990). The membrane is responsible for the selective inflow and outflow of molecules, ions, and water, and is a dynamic structure that performs a variety of functions. Membrane proteins carry out the dynamic process associated with membranes. Cellular

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membrane systems play an important role in the compartmentalization of cells and maintaining intercellular homeostasis. Stresses, such as dehydration, temperature abuse, radiation, and toxic chemicals can induce functional impairments to the cellular membrane systems through alterations to membrane physiochemcial properties such as activities of membrane-bound proteins, leading to loss of normal physiological processes, membrane leakage, and tissue injury. The common symptoms of the injury due to malfunction of membrane systems are water-soaked appearance, loss of turgor, leakage of electrolytes, and discoloration of tissues. The nutrients in leachates may also play a key role in the growth and development of pathogens (Garraway et al., 1989). Although plant tissues can re-absorb electrolytes, high doses of radiation may severely damage membrane and render plant tissue beyond repair.

Electrolyte leakage is generally considered as an indirect measure of plant cell membrane damage. The measurement requires readily available and inexpensive equipment, is not destructive of plant tissues, is easily used on diverse plant tissues, and is suitable for analyzing a large number of samples (Bajji et al., 2002). To measure electrolyte leakage, small pieces of samples are taken from plant tissues, and incubated in water or isotonic solutions. Many commercially prepared fresh-cut vegetables have already been cut into small pieces, and no further preparation is necessary. Therefore measurement of electrolyte leakage is ideal for commercially available fresh-cut vegetables. Irradiation increased electrolyte leakage in both mature green and pink tomatoes (El Assi et al., 1997), carrot discs (Skou, 1963), and muskmelon (Lester, 1989). Voisine et al. (1993) showed 2 kGy gamma radiation increased electrolyte leakage in cauliflower. Lester and Wolfenbarger (1990) found that electrolyte leakage is a good predictor of radiation injury on grapefruit flavedo tissue. Fan and Sokorai (2002) found that irradiation increased electrolyte leakage in fresh-cut Iceberg lettuce. Despite those studies, there has been no systematic measurement of electrolyte leakage in various fresh-cut vegetables.

Antioxidants can scavenge free radicals and therefore reduce the effects of radiation. Endogenous antioxidant capacity varies among vegetables (Cao et al., 1996). However, it is unclear whether the difference in antioxidant capacity between vegetables will impact their radiation sensitivities. The objectives of this study were to investigate the effect of irradiation on electrolyte leakage of various fresh-cut vegetables, to assess radiation sensitivity of vegetables using electrolyte leakage, and to study the correlation between the radiation sensitivity with endogenous antioxidants and phenolics content.

2. Materials and methods

2.1. Sample preparation

Thirteen fresh-cut vegetables were used including Romaine, Iceberg, red leaf, and green leaf lettuce (*Lactuca sativa* L.), cilantro (*Coriandrum sativum*), parsley (*Petroselinum hortense*), green onion (*Allium fistulosum*), carrot (*Daucus carota* L.), broccoli (*Brassica oleracea* L. var. italica Plen), endive (*Cichorium endiva* L.), red cabbage (*B. oleracea*, *Capitata group*), spinach (*Spinacia oleracea)*, and celery (*Apium graveolens*). These vegetables were chosen because of their economic importance or their association with contamination by food-borne pathogens and outbreaks of illnesses. The fresh-cut vegetables were either prepared at the laboratory from whole product or purchased as a fresh-cut product from local supermarkets. Lettuce, spinach and endive were cut with sharp stainless knives into 3 cm squares. Cilantro and parsley leaves were cut into ~2 cm long pieces. Fresh-cut broccoli was prepared using only florets without major stalks. Green onion and celery were cut into ~1 cm long pieces. The cut pieces were then rinsed with deionized water, drained and spin-dried using hand operated kitchen spinners (Wilton Industries Inc., Woodridge, IL). Shredded red cabbage and carrot were purchased and used without further preparation. All samples were placed into film bags which had been perforated with 4 holes (0.6 cm in diameter). There were four bags of samples for each treatment (dose), and each bag was treated as a replicate, and irradiated separately. Each bag contained 15 g of sample. The samples were then irradiated at 0, 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 kGy at $4 \pm 2\,^{\circ}C$. Electrolyte leakage was measured within 3 h after irradiation. Extra non-irradiated samples (10 g) were also stored at $-80\,^{\circ}C$ for antioxidant and phenolics analysis.

2.2. Irradiation and dosimetry

The samples were irradiated using a self-contained cesium-137 gamma radiation source (Lockheed Georgia Company, Marietta, GA) with an average dose rate of 0.096 kGy min^{-1}. Actual doses were 0.0, 0.48, 0.98, 1.46, 1.94, 2.49, 2.90 kGy with an average dose variation of 6.8%. Detailed description of irradiation and dosimetry was published earlier (Fan and Sokorai, 2002).

2.3. Measurement of electrolyte leakage

Five grams of each sample were incubated at 23 °C in 100 ml glass bottles containing 50–70 ml deionized water. During incubation, samples were agitated using a shaker (model M49125, Barnstead International, Dubuque, IA, USA) at a speed of 100 min^{-1}. Electrical conductivity of the bathing solution was measured at 1 min (C_1) and 60 min (C_{60}) of incubation using a CON 100 conductivity meter (Oakton, Singapore). The samples were then autoclaved (121 °C) for 25 min, and total conductivity (C_T) of bathing solution was then measured after cooling. Electrolyte leakage (E) was calculated from the following equation: $E = (C_{60} - C_1)/C_T \times 100$.

2.4. Antioxidant capacity

Samples (10 g) were homogenized with 20 ml 50% ethanol using a homogenizer (Virtishear, Virtis, Gardiner, NY) at a speed setting of 70 for 1 min. The homogenate was filtered through four-layer cheesecloth and then centrifuged at $12{,}000 \times g$ for 10 min at 5 °C in a Sorvall RC2-B refrigerated centrifuge (Kendro Laboratory Products, Newtown, CT). Antioxidant power in the supernatants was determined using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). Briefly, 100 μl samples were mixed with 3 ml FRAP reagent. The FRAP reagent was prepared fresh daily by combining 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,5,-tripyridyl-S-triazine in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio of 10:1:1 (v:v:v). The mixture was incubated at 23 °C for 30 min, then absorbance at 593 nm was measured with a spectrophotometer (Shimadzu UV-2402 spectrophotometer, Shimadzu Scientific Instruments, Columbia, MD). FRAP values were calculated from $FeSO_4$ standard curves, and expressed as μmol kg^{-1} FRAP values.

2.5. Phenolics analysis

Total phenolics content was measured using the Folin–Ciocalteu colorimetric method (Gao et al., 2000; Singleton et al., 1999). The extract (0.08 ml) used for FRAP assay, was mixed with 0.02 ml of 25 U ml^{-1} ascorbate oxidase, and then incubated at 23 °C for 90 min to remove ascorbic acid. The ascorbate-free extract was then mixed with 0.2 ml of Folin–Ciocalteu reagent (Sigma Chemical Co., St. Louis, MO), and incubated for 1 min at 23 °C. Then 3 ml of 5% Na_2CO_3 was added. Absorbances at 760 nm were recorded for the mixtures after 2 h incubation at 23 °C. Phenolic content was expressed as mg kg^{-1} gallic acid equivalent.

2.6. Statistical analysis

The experiments were repeated twice over a 6-month period. Four replicates were used per treatment in each experiment. The data obtained from the two experiments were similar and pooled. Data were subjected to statistical analysis using SAS version 6.12 (SAS Institute, Raleigh, NC, USA). The least significant difference (LSD) and Duncan's multiple range tests were performed using general linear models (GLM) procedure.

3. Results and discussion

Fig. 1 shows electrolyte leakage of various vegetables in response to radiation dose. In general, electrolyte leakage of all fresh-cut vegetables increased linearly ($P < 0.05$) with higher radiation dose. The R^2 values for most vegetables were above 0.85 while the R^2 values for broccoli and green leaf lettuce was the lowest (0.70 and 0.72, respectively). The results were similar to studies obtained earlier in tomato and muskmelon (El Assi et al., 1997; Lester, 1989).

There was variation among the vegetables in the rate of increase in electrolyte leakage in response to radiation dose (Table 1). The rate was the lowest for broccoli (0.19/kGy), and the highest for green onion (3.03/kGy). High rates of increase in electrolyte leakage as a func-

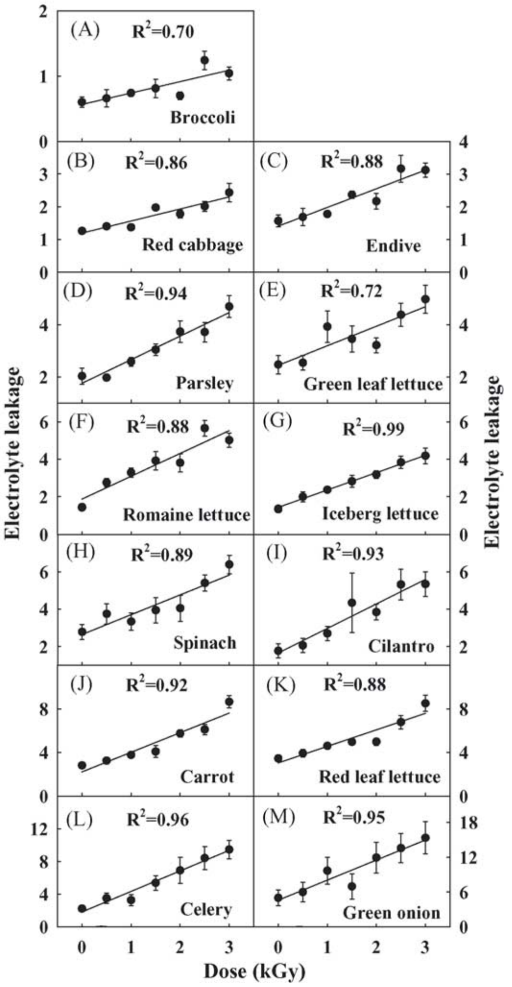

Fig. 1. Electrolyte leakage of fresh-cut vegetables as a function of radiation dose. Broccoli (A), red cabbage (B), endive (C), parsley (D), green leaf lettuce (E), Romaine lettuce (F), Iceberg lettuce (G), spinach (H), cilantro (I), carrot (J), red leaf lettuce (K), celery (L) and green onion (M) were irradiated with 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy gamma radiation at 5 °C. Electrolyte leakage was measured on the same day of irradiation. Vertical bars represent standard errors ($n = 8$).

tion of radiation dose suggest those vegetables were more sensitive to radiation damage. Although the rate of increase in electrolyte leakage can be used to assess relative radiation sensitivity and have been used to predict radiation injury in grapefruit peels (Lester and Wolfenbarger, 1990), it does not tell what radiation dose any individual vegetable can tolerate. To evaluate the radiation sensitivity of fresh-cut vegetables, a novel parameter, EL_{50} was introduced. EL_{50} was defined as radiation dose that increased electrolyte leakage by 50% over the non-irradiated controls. The rate of increase in electrolyte leakage and EL_{50} values were negatively correlated ($P < 0.002$). There was a large variation in EL_{50}, and EL_{50} values for most of the fresh-cut vegetables were between 0.6 and 1.4 kGy. There was no significant difference in EL_{50} among most vegetables due to large standard deviations. Broccoli, endive and red cabbage, however, had significantly ($P < 0.05$) higher EL_{50} values than cilantro, green onion and carrot when evaluated using LSD or Duncan's analysis (Table 1).

The antioxidant capacity expressed as FRAP values varied among the vegetables (Table 1). There was a 25-fold difference in FRAP values between red cabbage and celery. Red cabbage, cilantro, parsley, broccoli, and spinach had the highest FRAP values while Romaine and Iceberg lettuce, celery, and carrot had the lowest FRAP values, judged from LSD analysis and Duncan's multiple range test. An earlier study also found a large variation in antioxidant capacity between several common vegetables (Cao et al., 1996). The phenolics content of the vegetables had similar trends compared to FRAP values, and phenolics content and FRAP values were well correlated ($R^2 = 0.78$, $P < 0.01$). Phenolics are the major antioxidants in many vegetables. Therefore it is not a surprise that a strong correlation exists between antioxidant capacity and phenolics content.

The difference in EL_{50} values between the vegetables was relatively small compared to that in FRAP values and phenolic content. EL_{50} values had significant ($P = 0.02$) and slightly significant ($P = 0.053$) correlation with FRAP values and phenolic content, respectively. FRAP values and phenolic content had no significant ($P = 0.14$ and 13, respectively) correlation with the rate of increase in electrolyte leakage. Many early studies suggested that effects of radiation can be reduced by addition of antioxidants. For example, addition of several antioxidants including phenolics reduced irradiation-induced lipid oxidation in raw or cooked pork (Chen et al., 1999). Sato et al. (1995) suggested that antioxidants in plant tissues acting as radical scavengers protected the vacuolar membrane from radiation damage. Sommers et al. (2002) showed radiation resistance of the bacterium *Listeria monocytogenes* in cooked bologna was not affected by exogenous application of sodium erythorbate (an antiox-

Table 1
EL_{50}, rate of increase in electrolyte leakage, antioxidant activity and phenolics content of vegetables

Vegetables	EL_{50}[a] (kGy)	Leakage increase (kGy)	FRAP (μmol kg^{-1})	Phenolics (mg kg^{-1})
Red cabbage	2.00	0.40	28306	954
Broccoli	1.83	0.19	15221	962
Endive	1.44	0.52	6612	486
Spinach	1.30	1.08	14486	999
Red leaf lettuce	1.21	1.53	10865	713
Parsley	1.13	0.88	15538	1226
Green leaf lettuce	0.98	0.61	6750	476
Romaine lettuce	0.90	1.22	1723	179
Iceberg lettuce	0.89	0.94	1797	174
Cilantro	0.76	1.15	22667	1221
Green onion	0.69	3.03	8408	498
Carrot	0.62	1.81	2006	151
Celery	0.33	2.64	1126	82
$LSD_{0.05}$	0.66	0.63	3003	157

Thirteen fresh-cut vegetables were irradiated with 0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 kGy gamma radiation at 5 °C. EL_{50} and the rate of increase in electrolyte leakage were calculated from two experiments ($n = 8$).

[a] EL_{50} values are doses that increased electrolyte leakage by 50% over the non-irradiated controls.

idant) although sodium eryhthorbate increased radiation resistance of the bacterium in solution. Niemira et al. (2002) showed that the radiation sensitivity of *Escherichia coli* O157:H7 on different varieties of lettuce was not correlated with the endogenous antioxidants content. Our results indicate that the large difference in the endogenous antioxidant and phenolics content among the fresh-cut vegetables did not always make a difference in radiation sensitivity of vegetables as measured by electrolyte leakage.

The location and availability of the endogenous antioxidants may limit the scavenging ability of antioxidants against radiation damage. Although antioxidants can be present in lipid-soluble form and in bond form in the cell wall, most water-soluble antioxidants such as phenolics are located in vacuoles of plant tissues (Harborne, 1989). Ionizing radiation exerts its effect through free radicals generated mainly from radiolysis of water, and the free radicals attack membrane components, resulting in increased permeability. Therefore, to protect the electrolyte leakage of membrane caused by irradiation, antioxidant compounds need to embedded or be near the membranes to protect against free radicals damage. The antioxidants measured in the study are water/ethanol soluble compounds, therefore, lipid-soluble and water-soluble antioxidants were not distinguished. It may be interesting to see whether there is a correlation between a particular group of antioxidants and radiation sensitivity.

Using the EL_{50} values defined in the present study, our results suggest that most of the vegetables tolerated a radiation dose of 0.6–1.4 kGy, which appears to be in agreement with our earlier observations on Iceberg lettuce (Fan and Sokorai, 2002), green onion (Fan et al., 2003a), and cilantro (Fan et al., 2003b) showing these vegetables treated with 1.0 kGy radiation had acceptable sensory and nutritional quality.

Our results showed irradiation induced increases in electrolyte leakage in all vegetables, and the sensitivity (EL_{50}) to irradiation varied among vegetables. Therefore, measurement of electrolyte leakage may be used for the assessment of membrane damage and radiation sensitivity of various vegetables. However, EL_{50} values were not correlated to shelf-life or post-storage visual quality changes of irradiated vegetables (data not shown). Visual quality assessed after 14 days of post-irradiation storage at 3 °C suggested that all vegetables can tolerate at least 1 kGy radiation (data not shown). Of course, other quality attributes in addition to visual quality, can limit the acceptability of irradiated vegetables. Many factors other than membrane permeability can influence quality attributes. Quality losses (such as browning) can be a result of chemical changes not necessarily related to membrane permeability. Irradiation increases phenolic content in vegetables (Qufedjikh et al., 2000; Fan and Sokorai, 2002), which may in turn influence appearance, flavor and nutritive values. Irradiation can inactivate spoilage microorganisms present in vegetables, resulting in extended shelf-life of vegetables. The EL_{50} values calculated from this study would not predict the quality loss due to decay. Furthermore, post-irradiation conditions such as maturity of produce, storage temperature and headspace atmosphere, can influence shelf-life and quality parameters of vegetables. The radiation sensitivity measured using electrolyte leakage should be used only as indicators of potential post-storage quality.

There was a variation in radiation sensitivity among the vegetables judged from EL_{50}. The variation may be due to differences in lipid composition, or other factors such as cell wall structure, cuticle thickness, and chemical (antioxidants) composition. It is interesting to notice that two of the most radioresistant vegetables, broccoli and red cabbage, belonging to the same species (*B. oleracea*) of cruciferous plants, had high antioxidant capacity. Difference in chilling-induced electrolyte leakage between different genotypes of tomato has been observed (van de Dijk et al., 1985).

It should be pointed out that there are limitations in this study. While the produce was purchased in local markets with marketable quality, the source and age of the materials were unknown. Maturity and age of the raw materials may affect the response of samples to irradiation. Also, electrolyte leakage should be measured on the day of irradiation as did in the present study. After 2 weeks storage at 3 °C, electrolyte leakage of non-irradiated vegetables was often found to be higher than irradiated ones (data not shown), presumably due to higher rates of senescence and deterioration of the non-irradiated vegetables as a result of microbial activity.

In summary, this study employed electrolyte leakage as a means of assessing radiation sensitivity of 13 fresh-cut vegetables, and a novel parameter was introduced to evaluate the radiation resistance of fresh-cut vegetables. The results suggest that radiation resistance varied among the vegetables. Broccoli, endive, and red cabbage had higher radiation resistance than cilantro, green onion and carrot. Radiation resistance was not related to the endogenous antioxidant capacity or phenolics content.

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